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Bioreactivity: Studies on a simple brain stem reflex in behaving animals.

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Professor Barry L. Jacobs

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A major problem in attempting to understand complex physiological processes, such as brain neuromodulation, or complex behavioral processes, such as arousal, is finding a simple system that will permit such analyses. The brain stem masseteric (jaw closure) reflex in cats is such a system. It is simple, containing only one synapse in brain, and receives dense inputs from two neurochemical systems important in neuromodulation and arousal. Initial pharmacologic studies showed that locally applied norepinephrine facilitated the reflex response. More importantly, physiologic conditions, known to activate the brain norepinephrine system, also facilitated the response. This latter finding was shown to be causal, rather than correlative, by a study which found that the facilitation could be blocked by prior destruction of the norepinephrine input specifically to the reflex circuitry. These data represent the first definitive example of an activational effect in an intact and behaving organism being attributable to a particular central neurotransmitter acting at a specific brain site.

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1. RESEARCH OBJECTIVES

AFOSR-TR. 88 - 0696

The neural substrates of mammalian bioreactivity are undoubtedly very complex. Nevertheless, a large body of research points to the system of brain noradrenergic (NE) neurons as playing a central role in bioreactivity. Much of this evidence, however, remains indirect, largely because of the inherent difficulties involved in examining brain activity in a precise fashion, under physiological conditions, and without simultaneously perturbing the system under study. One means of accomplishing such analyses is to examine single unit activity (extracellularly recorded action potentials) of NE neurons in behaving animals. For the past four years, largely through funding from the AFOSR, we have been studying the single unit activity of brain NE neurons in the area of the locus coeruleus (LC) in unanesthetized and unrestrained cats. The results of our work, in conjunction with that of other investigators in this field, provide strong support for the role of NE neurons in various aspects of bioreactivity, including stress. This work constitutes a significant and necessary step in research directed at understanding the neural substrates of bioreactivity, but it is limited in two important ways. First, studies of single unit activity in conjunction with physiology and behavior are only correlative. Second, the degree to which single unit activity is related directly and linearly to the amount of synaptic release of neurotransmitter is an open question.

Experiments in this grant are aimed at two issues. First, we propose to examine the cause and effect relationship between NE and bioreactivity, as reflected in changes in the masseteric reflex. This system is an ideal one for such analyses because it is a simple reflex (monosynaptic) whose motor component receives a dense noradrenergic input. (It also receives a dense serotonergic (5-HT) input and we propose to compare and contrast its functional influence with that of NE.) Second, we propose to measure directly neurotransmitter release in behaving animals through the use of in vivo dialysis probes. These measures will be carried out in the motor nucleus component (motor V; Mo V) of the masseteric reflex arc, and will be related quantitatively to simultaneous changes in the single unit activity of NE (or 5-HT) neurons innervating Mo V and to simultaneous changes in the magnitude of the elicited masseteric reflex response.

The primary dependent variable in these studies, the masseteric reflex, is not simply a randomly chosen piece of behavior. Jaw closing (or clenching) is a well known response to stress and a component of the anxiety syndrome. Experimental evidence from studies in humans directly demonstrates that the masseteric reflex response is augmented by fear or anxiety. Thus, the masseteric reflex represents a simple behavior having direct relevance to bioreactivity. (AU)

A series of four groups of studies are proposed which will elucidate the functional role of NE (and to some extent 5-HT) in modulating bioreactivity, as reflected in changes in the amplitude of the masseteric reflex response, in unrestrained and unanesthetized cats. 1. Examine the direct effects of NE or 5-HT upon the masseteric reflex through the use of local injections of NE, 5-HT and their agonists or antagonists (in some cases the antagonists will be administered systemically). We also plan to manipulate the NE and 5-HT inputs to Mo V by locally infusing drugs known

to directly affect the activity of the neurons that give rise to these inputs. By combining these latter studies with specific neurotoxin induced denervation, we will also attempt to demonstrate that there is a cause and effect relationship between changes in NE or 5-HT unit activity and changes in the masseteric reflex. Finally, by local administration of NE or 5-HT drugs in combination with local administration of drugs affecting the "other" system, we hope to get some indication of the interaction between these two neurochemical systems which influence the masseteric reflex. 2. By examining the jaw opening response (digastric reflex) under the same experimental conditions as the jaw closure response (masseteric reflex) we will address the question of whether NE produces global or selective response facilitation. 3. Under conditions presumed to influence NE neurotransmission (e.g. white noise presentation), simultaneously measure NE single unit activity, in vivo release of NE in Mo V, and the amplitude of the masseteric reflex. If time allows, we also propose to do a parallel series of studies for 5-HT. 4. Determine, through double labelling studies employing retrograde tracers and immunocytochemistry, the site of origin of the dense NE and 5-HT inputs to Mo V in the cat.

2. STATUS OF THE RESEARCH

Work has begun on all four of the aforementioned objectives. Objectives 2 and 4 are in very early stages and there are no substantive data to report, therefore, I will focus on objectives 1 and 3.

The heart of this proposal was an attempt to examine directly the role of NE or 5-HT on the masseteric reflex. When NE (0.125 - 0.5 μ g in 0.5 μ l) is injected directly into Mo V of the cat, we see a consistent, dose-dependent facilitation of the reflex. Pharmacologic analyses have shown that this is an action at an alpha-1 adrenergic receptor. When we examine the effects of a number of manipulations known to activate the brain NE system, such as exposure to loud white noise, confrontation with a dog, and phasic presentation of a click, we see facilitation or augmentation of the reflex response. This relationship is, however, only correlative. Therefore, in the culmination of this experiment, we repeated these studies in animals that had received unilateral denervation of the NE input to Mo V. The reflex response on the denervated side of the brain was then compared to the reflex response on the intact side of the brain. In strong support of our hypothesis, local NE denervation with the catecholamine selective neurotoxin 6-OHDA, either markedly attenuated or blocked the environmentally-induced activation of the reflex. We believe this is the first direct evidence of a facilitatory role for NE in brain, examined under "physiologically relevant" conditions in an intact and behaving animal.

The pharmacologic results for 5-HT are similar to those for NE, although we have not yet specified the receptor subtype where 5-HT acts upon the reflex. Activating the brain 5-HT system under physiological conditions is something that we have not yet accomplished because our electrophysiological studies have not yet uncovered the conditions for doing this.

Our third objective was to examine the release of NE and/or 5-HT in Mo V using in vivo dialysis. The objective would be to relate directly the



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release of neurotransmitter to changes in the reflex response. To date, we have been successful in measuring brain 5-HT using in vivo brain microdialysis, but we have not yet examined this directly in Mo V.

3. PUBLICATIONS

In addition to the work on the masseteric reflex, a portion of the AFOSR funds has been used for supporting our single unit studies of LC-NE neurons. To date, our masseteric reflex work has appeared only in abstract form.

- Abercrombie, E.D. and Jacobs, B.L. Single unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. I. Acutely presented stressful and non-stressful stimuli. Journal of Neuroscience, 1987, 7, 2837-2843.
- Abercrombie, E.D. and Jacobs, B.L. Single unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. II. Adaptation to chronically presented stressful stimuli. Journal of Neuroscience, 1987, 7, 2844-2848.
- Morilak, D.A., Fornal, C. and Jacobs, B.L. Effects of physiological manipulations on locus coeruleus neuronal activity in freely moving cats: I. Thermoregulatory challenge. Brain Research, 1987, 422, 17-23.
- Morilak, D.A., Fornal, C. and Jacobs, B.L. Effects of physiological manipulations on locus coeruleus neuronal activity in freely moving cats: II. Cardiovascular challenge. Brain Research, 1987, 422, 24-31.
- Morilak, D.A., Fornal, C. and Jacobs, B.L. Effects of physiological manipulations on locus coeruleus neuronal activity in freely moving cats: III. Glucoregulatory challenge. Brain Research, 1987, 422, 32-39.
- Morilak, D.A., Stafford, I.L. and Jacobs, B.L. The masseteric reflex: a model system for studying monoaminergic neurotransmission in behaving animals. Soc. Neurosci., Abst., 1987, 13, 659.
- Stafford, I.L. and Jacobs, B.L. Noradrenergic facilitation of the masseteric reflex in behaving animals. Soc. Neurosci., Abst., 1987, 13, 659.
- Stafford, I.L. and Jacobs, B.L. Modulation of the masseteric reflex by environmental conditions. Soc. Neurosci., Abst. (in press).
- Jacobs, B.L. and Stafford, I.L. Modulation of the masseteric reflex by brain norepinephrine. Soc. Neurosci., Abst. (in press).
- Ribiero-do-Valle, L.E., Metzler, C.W., Stafford, I.L. and Jacobs, B.L. Serotonergic facilitation of the masseteric reflex in behaving cats. Soc. Neurosci., Abst. (in press).

4. PROFESSIONAL PERSONNEL

Professor Barry Jacobs
Dr. Luiz Eduardo Ribiero-do-Valle
Ms. Chris Metzler
Ms. Ines Stafford

5. INTERACTIONS (spoken papers)

a. Seminars by Barry L. Jacobs

Robert Wood Johnson Medical School
Mid-Atlantic Chapter of ASPET
All India Medical School (New Delhi)
New York University
New Jersey Society for Neuropharmacology
Albany Medical College
Department of Pharmacology, University of Cagliari (Italy)
Massachusetts Institute of Technology
U.S.V.A. Hospital (Sepulveda, CA)

b. Meeting Presentations

Invited Speaker - International Catecholamine Meeting,
Jerusalem, Israel

Invited Participant - 4th Symposium on "Catecholamines and
Other Neurotransmitters in Stress, Smolenice Castle
Czechoslovakia

Invited Speaker - 5th International Congress of Sleep
Research, Copenhagen, Denmark

Invited Speaker - Bioreactivity Conference, U.S. Air Force,
San Antonio, Texas

6. NEW DISCOVERIES

None

7. OTHER STATEMENTS

None